UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/082,772	02/25/2002	Peter Droge	DEBE:008US	4391

7590 02/21/2008

Steven L. Highlander
FULBRIGHT & JAWORSKI L.L.P.
Suite 2400
600 Congress Avenue,
Austin, TX 78701

EXAMINER	
NGUYEN, QUANG	
ART UNIT	**PAPER NUMBER**
1633	

MAIL DATE	DELIVERY MODE
02/21/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/082,772	DROGE ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 December 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *29,30,32-39,43-51 and 58* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *29,30,32-39,43-51 and 58* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on 12/03/07 was entered.

It is noted that Applicants elected previously **without traverse** of Group I, drawn to a method of sequence specific recombination of DNA in a eukaryotic cell, **wherein the method is performed in a cell culture (or *ex vivo*)**, in the Amendment filed on 8/29/03.

Amended claims 29-30, 32-39, 43-51 and 58 are pending in the present application, and they are examined on the merits herein.

Response to Amendment

The rejection under 35 U.S.C. 112, first paragraph, for New Matter was withdrawn in light of Applicant's amendment and arguments as set forth in the amendment filed on 8/27/07 (pages 6-7).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein

were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

. Claims 29-30, 32-33, 36, 38, 44-48 and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999; IDS) for the same reasons already set forth in the Office action mailed on 4/17/07 (pages 4-7). ***The same rejection is restated below.***

Crouzet et al teach a method for producing therapeutic DNA molecules by excision from a plasmid or from a chromosome (must be stably integrated) by site-specific recombination, wherein the site-specific recombination is carried out in a host cell or *in vitro*, and wherein the site-specific recombination is carried out by means of various systems which lead to site-specific recombination between sequences (col. 3, line 27 continues to line 20 of col. 4; Figures 1-2). Specifically, Crouzet et al disclose that the gene or genes of interest flanked by the two sequences permitting site-specific recombination, positioned in the direct orientation in a vector, a phage or a virus, and the specific recombination sequences and recombinases used can belong to different structural classes, including the integrase family of bacteriophage λ and the attP and attB sequences catalyzed by the phage lambda integrase (col. 4, lines 21-52; col. 5, lines 32-41). The disclosed attB sequence is identical to SEQ ID NO:1 of the present

application (see SEQ ID NO:6), and therefore the corresponding attP recombination sequence must have the same SEQ ID NO: 2 of the present application, or at least a derivative thereof. Additionally, Crouzet et al. teach that **their genetic constructs can be used for the construct of cell hosts having disclosed elements integrated in their genomes** (col. 4, lines 4-9; col. 8, lines 64; col. 9, lines 27-60; and Figure 1). Crouzet et al further teach that the recombinase to be introduced into a host cell can be under the control of a promoter or a system of inducible promoters, and it can be carried by a plasmid, a phage or even in the non-therapeutic region of the recombinant DNAs (col. 7, line 45 continues to line 39 of col. 8), and that **any type of host cell can be used including eukaryotic cells such as yeasts, animal cells, plant cells, mammalian cells such as CHO, COS and mouse NIH3T3** (col. 9, lines 48-60). Crouzet et al also disclose that their genetic constructs comprise one or more genes of interest such as the genes coding for enzymes, hormones, growth factors, apolipoproteins, tumor suppressor genes, suicide genes, natural or artificial immunoglobulin or an antigenic peptide (col. 13, lines 14-50).

Crouzet et al do not specifically teach the use of any modified λ Integrase, specifically Int-h or Int h/218 in their method of producing therapeutic DNA molecules, even though they teach that any specific recombination sequences and appropriate recombinases can be used, including those belonging to the bacteriophage λ system.

However, at the effective filing date of the present application Christ & Droge already taught the mutant λ Integrases, Int-h and Int-h/218, that are capable of mediating substrate deletion through λ site-specific recombination in prokaryotes in the

absence of co-factors Fis and Xis (see at least the abstract; page 829, right hand column, top and bottom of the first full paragraph). Christ & Droge further teach that the potential of these mutants to perform recombination in the absence of accessory factors is due to their enhanced affinity for core sites, and that Int-h is known to recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type Integrase (page 827, left column, second full paragraph; page 828, left column, top of last paragraph).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the method taught by Crouzet et al by utilizing specifically the mutant λ Integrases, Int-h and Int-h/218, of Christ & Droge in their method of producing therapeutic DNA molecules due to the advantages offered by the mutant integrases, at least the mutant Integrases can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than the wild-type Intergrase.

An ordinary skilled artisan would have been motivated to carry out the above modification because as taught by Christ & Droge, at least these mutant Integrases can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type Integrase.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Christ & Droge, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments with respect to the above rejection in the Amendment filed on 8/27/07 (pages 8-10) have been fully considered but they are respectfully not found persuasive.

Once again, Applicants argued that Crouzet et al use exclusively the **wild-type** lambda integrase, while Christ & Droge relates exclusively to integrative and excisive attL/attR and attP/attB recombination performed in **prokaryotes**. Applicants emphasized that there was no motivation for combining these two very distinct systems, and even if there was, there was no likelihood of success that they would be compatible, i.e., that the modified integrases of Christ & Droge would function in a eukaryotic system. Applicants further argued that the above rejection was based on hindsight and that the Examiner dismissed evidence provided by Applicants in the form of the Declaration filed under 37 CFR 1.132 on 10/04/06 even in light of amended claims filed on 3/13/07.

Firstly, Crouzet et al already taught a method for producing therapeutic DNA molecules by excision from a plasmid or from a chromosome by site-specific recombination, wherein the site-specific recombination is carried out in a host cell (including eukaryotic cells) or *in vitro*, and wherein the site-specific recombination is carried out by means of various systems which lead to site-specific recombination between sequences, including the use of specific recombination sequences and appropriate recombinases belonging to different structural classes, including the

integrase family of bacteriophage λ and the attP and attB sequences catalyzed by the phage lambda integrase. In addition, Christ & Droge already taught the mutant λ Integrases, Int-h and Int-h/218, that are capable of mediating substrate deletion through λ site-specific recombination in prokaryotes in the absence of co-factors Fis and Xis; and that the potential of these mutants to perform recombination in the absence of accessory factors is due to their enhanced affinity for core sites, and that Int-h is known to recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type Integrase. As already set forth in the above rejection, an ordinary skilled artisan would have been motivated to modify the method taught by Crouzet et al by also utilizing the mutant λ Integrases, Int-h and Int-h/218, of Christ & Droge in their method of producing therapeutic DNA molecules due to the advantages offered by these mutant integrases. For example, at least these mutant Integrases can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than the wild-type Intergrase, and the potential of these mutants to perform recombination in the absence of accessory factors is due to their enhanced affinity for core sites. Thus, there are clear motivations to combine the teachings of Crouzet et al. and Christ & Droge.

Secondly, with respect to the issue of reasonable expectation of success it should be noted that since wild-type lambda integrases are capable of mediating sequence specific recombination events **in both eukaryotic and prokaryotic cells**, and it is known in the prior art that the conditions required by a wild-type lambda integrase to mediate recombination reactions in prokaryotic cells, under physiological

conditions and *in vitro* conditions are apparently more stringent than those required by the Int-h as evidenced at least by the teachings of Hartley et al., Christ & Droge and Lange-Gustafson et al of record, it is therefore reasonable for an ordinary skilled artisan to expect that at least Int-h or both Int-h and Int-h/218 are also able to function in eukaryotic cells.

Thirdly, the Declaration filed under 37 CFR 1.132 on 10/04/06 was considered by the Examiner but it was not found to overcome the rejection of record. Paragraph 3 of the Declaration contains the following statements with respect to the Int-h/218 mutant "It was, therefore, not clear whether this enzyme would be active in the absence of protein co-factors and negative DNA supercoiling of substrate DNA. However, both factors are present in *E. Coli.* Before we transferred this mutant to mammalian cells, we knew that the enzyme could catalyze an abnormal reaction in *E.coli* in the absence of the co-factor IHF (Christ and Droge, 1999). However, one has to realize that DNA substrates (whether episomal or genomic) are negatively supercoiled inside *E. Coli.* It was, therefore, not obvious to one of ordinary skill to deduce from the existing data that the mutant recombinase would work inside mammalian cells where the DNA is topologically relaxed. In fact, up to this day, the reason why both Int-h and the double mutant Int-h/218 are functional in eukaryotic cells remains a mystery. One possibility is that there is an unidentified mammalian co-factor which supports the prokaryotic recombinase." Apart from the noted difference that eukaryotic DNA is topologically relaxed while *E. Coli* DNA is negatively supercoiled, the Declaration does not provide any factual evidence why an ordinary skilled artisan at the time the invention was made would not

have a reasonable expectation that Int-h and/or Int-h/218 are not capable of mediating a sequence-specific recombination in eukaryotic cells. On the contrary to Applicants' position, since wild-type lambda integrases are capable of mediating sequence specific recombination events **in both eukaryotic (DNA substrates are topologically relaxed) and prokaryotic cells (DNA substrates are negatively supercoiled)**, and it is known in the prior art that the conditions required by a wild-type lambda integrase to mediate recombination reactions in prokaryotic cells, under physiological conditions and *in vitro* conditions are apparently more stringent than those required by the Int-h as evidenced at least by the teachings of Hartley et al., Christ & Droge and Lange-Gustafson et al of record, it is entirely reasonable for an ordinary skilled artisan to expect that at least Int-h or both Int-h and Int-h/218 are able to function in eukaryotic cells.

Accordingly, claims 29-30, 32-33, 36, 38, 44-48 and 58 are still rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. in view of Christ & Droge (for the same reasons already set forth above.

Amended claims 29 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999; IDS) as applied to claims 29-30, 32-33, 36, 38, 44-48 and 58 above, and further in view of Capecchi et al. (US 5,464,764)) for the same reasons already set forth in the Office action mailed on 4/17/07 (pages 8-10). ***The same rejection is restated below.***

The teachings of Crouzet et al. and Christ & Droge have been discussed above. However, neither Crouzet et al nor Christ & Droge teach that the first and/or the second DNA segment further comprising a sequence effecting integration of said first and/or second DNA segment into the genome of a cell by homologous recombination, even though Crouzet et al teach specifically that the genetic constructs can be used for the construct of cell hosts having disclosed elements integrated in their genomes and that the insertion may also be carried out by homologous recombination (col. 4, lines 4-9; col. 8, lines 60-64; and Figure 1).

However, at the effective filing date of the present application Capecchi et al. already describe a well-known method and a positive-negative selector vectors comprising sequences to be introduced in the genome of a target cell by the homologous recombination approach (see abstract and Figure 1).

It would have been obvious for an ordinary skilled artisan to further modify the modified method of Crouzet et al and Christ & Droge by introducing the genetic construct in the form of a positive-negative selector vector described by Capecchi et al. into a target host cell capable of homologous recombination to incorporate their genetic construct in the genome of the host cell as specifically taught by Crouzet et al.

An ordinary skilled artisan would have been motivated to carry out the above modification because the approach taught by Capecchi et al. overcomes many problems associated with random integration, and that the gene of interest can be introduced into any predetermined region of the genome of a target host cell.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Christ & Droge and Capecchi et al, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments with respect to the above rejection in the Amendment filed on 8/27/07 (pages 10-11) have been fully considered but they are respectfully not found persuasive.

Once again, Applicants argued that Capecchi et al failed to remedy the lack of motivation and the lack of an expectation of success for combining the Crouzet et al. and Christ & Droge references for the reasons set forth in Applicants' arguments in the above rejection for claims 29-30, 32-33, 36, 38, 44-48 and 58.

Please refer to the above Examiner's response to Applicants' arguments on the lack of motivation and the lack of an expectation of success for combining the Crouzet et al. and Christ & Droge references. The teachings of Capecchi et al were used to supplement the combined teachings of Crouzet et al. and Christ & Droge on the limitation recited in dependent claim 43.

Amended claims 29, 34-35, 36-37 and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge

(J. Mol. Biol. 288:825-836, 1999; IDS) as applied to claims 29-30, 32-33, 36, 38, 44-48 and 58 above, and further in view of Hartley et al. (US 5,888,732) for the same reasons already set forth in the Office action mailed on 4/17/07 (pages 10-12). ***The same rejection is restated below.***

The teachings of Crouzet et al. and Christ & Droge have been discussed above. However, neither Crouzet et al nor Christ & Droge teach that the first DNA segment stably integrated into the genome of a eukaryotic cell comprising an attL sequence according to SEQ ID NO:3 or a derivative thereof, or an attR sequence according to SEQ ID NO:4 or a derivative thereof, even though Crouzet et al teach specifically that a method for producing therapeutic DNA molecules by excision from a plasmid or from a chromosome (must be stably integrated) by site-specific recombination, wherein the site-specific recombination is carried out in a host cell or *in vitro*, and wherein the site-specific recombination is carried out by means of various systems which lead to site-specific recombination between sequences, including the use of bacteriophage λ and the attP and attB sequences (col. 3, line 27 continues to line 20 of col. 4; Figures 1-2).

However, at the effective filing date of the present application Hartley et al already taught both *in vitro* and *in vivo* (e.g., in eukaryotic host cells) methods for the exchange of DNA segments through the use of various recombination proteins described in the art, including λ Integrase (see at least Summary of the Invention; col. 13, lines 35-55 and line 57 continues to line 24 of col. 16) and its recognition sequences including attB, attP, attL, and attR sequences (col. 8, lines 43-63). The disclosed attL and attR recombination sequences that are catalyzed by λ Integrase taught by Hartley

et al. must have the same SEQ ID NO:3 and SEQ ID NO:4, respectively, or at least a derivative thereof. Hartley et al. also teach the use of integrase in the presence of λ protein Xis (excise) to catalyze the reaction of attR and attL (col. 15, lines 1-3). Hartley et al. also teach engineered att recombination sites having one or multiple mutations to enhance specificity or efficiency of the recombination reaction, and that a tripartite protein fusion could be accomplished with parental plasmids containing recombination sites attR1 and attR2; attL1 and attL3; and attR3 and attL2 (col. 16, lines 26-61; col. 17, line 47 continues to line 46 of col. 18).

It would have been obvious for an ordinary skilled artisan to modify the method of Crouzet et al and Christ & Droge by also utilizing the λ Integrase recognition sequences attL and attR sequences flanking the gene or genes of interest, optionally along with a source of Xis factor under the control of a promoter or a system of inducible promoters, for the production of therapeutic DNA molecules. Once again, it is noted that Crouzet et al teach specifically that the site-specific recombination is carried by means of various systems which lead to site-specific recombination between sequences.

An ordinary skilled artisan would have been motivated to carry out the above modification because the excision system involving λ Integrase and its recognition sites attL and attR sequences is well known and already taught by Hartley et al for the exchange of DNA segment in both *in vitro* and *in vivo*.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Christ & Droge and Hartley et al, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments with respect to the above rejection in the Amendment filed on 8/27/07 (page 11) have been fully considered but they are respectfully not found persuasive.

Once again, Applicants argued that Hartley et al failed to remedy the lack of motivation and the lack of an expectation of success for combining the Crouzet et al. and Christ & Droge references for the reasons set forth in Applicants' arguments in the above rejection for claims 29-30, 32-33, 36, 38, 44-48 and 58.

Please refer to the above Examiner's response to Applicants' arguments on the lack of motivation and the lack of an expectation of success for combining the Crouzet et al. and Christ & Droge references. The teachings of Hartley et al were used primarily to supplement the combined teachings of Crouzet et al. and Christ & Droge on limitations recited in dependent claims.

Claims 29 and 49-51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Calos (US 6,632,672), Hartley et al. (US 5,888,732) and Christ & Droge (J. Mol. Biol. 288:825-836, 1999; IDS). ***This is a new ground of rejection.***

Crouzet et al teach a method for producing therapeutic DNA molecules by excision from a plasmid or from a chromosome (integrated into a cellular genome) by site-specific recombination, wherein the site-specific recombination is carried out in a host cell or *in vitro*, and wherein the site-specific recombination is carried out by means of various systems which lead to site-specific recombination between sequences (col. 3, line 27 continues to line 20 of col. 4; Figures 1-2). Specifically, Crouzet et al disclose that the gene or genes of interest flanked by the two sequences permitting site-specific recombination, positioned in the direct orientation in a vector, a phage or a virus, and the specific recombination sequences and recombinases used can belong to different structural classes, including the integrase family of bacteriophage λ and the preferred attP and attB sequences catalyzed by the phage lambda integrase (col. 4, lines 21-52; col. 5, lines 32-41). The disclosed attB sequence is identical to SEQ ID NO:1 of the present application (see SEQ ID NO:6), and therefore the corresponding attP recombination sequence must have the same SEQ ID NO: 2 of the present application, or at least a derivative thereof. Additionally, Crouzet et al. teach that **their genetic constructs can be used for the construct of cell hosts having disclosed elements integrated in their genomes** (col. 4, lines 4-9; col. 8, lines 64; col. 9, lines 27-60; and Figure 1). Crouzet et al further teach that the recombinase to be introduced into a host cell can be under the control of a promoter or a system of inducible promoters, and it can be carried by a plasmid, a phage or even in the non-therapeutic region of the recombinant DNAs (col. 7, line 45 continues to line 39 of col. 8), and that **any type of host cell can be used including eukaryotic cells such as yeasts, animal cells,**

plant cells, mammalian cells such as CHO, COS and mouse NIH3T3 (col. 9, lines 48-60). Crouzet et al also disclose that their genetic constructs comprise one or more genes of interest such as the genes coding for enzymes, hormones, growth factors, apolipoproteins, tumor suppressor genes, suicide genes, natural or artificial immunoglobulin or an antigenic peptide (col. 13, lines 14-50).

Crouzet et al do not teach specifically how the therapeutic DNA molecules are integrated in a eukaryotic cellular genome to be excised later by site-specific recombination, particularly the use of any modified λ Integrase (e.g., Int-h or Int h/218) even though they teach that any specific recombination sequences and appropriate recombinases can be used, including those belonging to the bacteriophage λ system; or the process involving performing a second sequence specific recombination of DNA by Int-h or Int-h/218 and a Xis factor.

However, at the effective filing date of the present application Calos already taught a method of specifically integrating a polynucleotide sequence of interest in a genome of a eukaryotic cell (yeasts, mammalian host cells) using a targeting construct comprising a i) first recombination site and a polynucleotide sequence of interest, and ii) a site-specific recombinase, and the genome of the eukaryotic cell comprises a second recombination wherein recombination between the first and second recombination sites is facilitated by the site-specific recombinase, based in part on the discovery that there exist in various eukaryotic genomes specific nucleic acid sequences (pseudo-recombination sequences) that may be distinct from wild-type recombination sequences and that can be recognized by a site specific recombinase (see at least the abstract;

col. 13, line 60 continues to line 16 of col. 14; col. 23, line 10 continues to line 18 of col. 24). Calos et al further disclosed that the used site-specific recombinase encompasses Cre, λ integrase and others (col. 7, lines 33-43; col. 14, lines 17-45); and that either wild-type attB or wild-type attP sequence can be used in the targeting construct (col. 18, line 58 continues to line 54 of col. 19).

Additionally, at the effective filing date of the present application Hartley et al also taught both *in vitro* and *in vivo* (e.g., in eukaryotic host cells) methods for the exchange of DNA segments through the use of various recombination proteins described in the art, including λ Integrase (see at least Summary of the Invention; col. 13, lines 35-55 and line 57 continues to line 24 of col. 16) and its recognition sequences including attB, attP, attL, and attR sequences (col. 8, lines 43-63). Hartley et al. disclosed that integrative recombination involves the Int and IHF proteins and sites attP and attB, and recombination results in the formation of two new sites attL and attR; while excisive recombination requires Int, IHF, and Xis, and sites attL and attR to generate att P and attB (col. 14, line 13 continues to line 4 of col. 15). Hartley et al. also taught engineered att recombination sites having one or multiple mutations to enhance specificity or efficiency of the recombination reaction, and that a tripartite protein fusion could be accomplished with parental plasmids containing recombination sites attR1 and attR2; attL1 and attL3; and attR3 and attL2 (col. 16, lines 26-61; col. 17, line 47 continues to line 46 of col. 18).

Furthermore, Christ & Droge also taught the mutant λ Integrases, Int-h and Int-h/218, that are capable of mediating substrate deletion through λ site-specific

recombination in prokaryotes in the absence of co-factors Fis and Xis (see at least the abstract; page 829, right hand column, top and bottom of the first full paragraph). Christ & Droge further teach that the potential of these mutants to perform recombination in the absence of accessory factors is due to their enhanced affinity for core sites, and that Int-h is known to recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type Integrase (page 827, left column, second full paragraph; page 828, left column, top of last paragraph).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the method taught by Crouzet et al by using a eukaryotic cell host whose genome comprises an integrated expression cassette containing a gene(s) of interest flanked by the two sequences permitting site-specific recombination such as attL and attR as a result of integrating a targeting construct containing either wild-type attB or wild-type attP sequence as the first recombination site and a gene(s) of interest into the eukaryotic host cell whose genome already containing either pseudo attP or pseudo attB, respectively, in light of the teachings of Calos; and the subsequent release of the integrated expression cassette containing a gene(s) of interest from these recombinant eukaryotic hosts by a second recombination in the presence of a Xis factor, including the Xis factor under the control of a promoter or a system of inducible promoters, and the used recombinases are λ Integrases mutants Int-h and Int-h/218 in light of the teachings of Hartley et al. and Christ & Droge as discussed above.

An ordinary skilled artisan would have been motivated to carry out the above modifications because at least the method of site-specifically integrating a

polynucleotide sequence of interest in a genome of a eukaryotic cell using a site-specific recombinase has been well established by Calos, along with the well-characterized λ Integrase recombinase system used for exchange DNA segments in both *in vitro* and *in vivo* as taught by Hartley et al. Additionally, Christ & Droge taught at least that the mutant λ Integrases, Int-h and Int-h/218, can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type λ Integrase, particularly suitable for performing recombinations between wild-type att sites and corresponding pseudo att sites. Similar to the expression of the recombinase under the control of a promoter or a system of inducible promoters, the expression of the X factor under the control of a system of inducible promoter is also highly desirable for a better regulation of the excision reaction in the recombinant eukaryotic host cells.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Calos, Hartley et al., and Christ & Droge, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

QUANG NGUYEN, PH.D.
PRIMARY EXAMINER